

NEOGEN
REVEAL Q+ FOR ZEARELENONE
USING ACCUSCAN III AND ACCUSCAN GOLD READERS

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GENERAL INFORMATION

The Reveal Q+ for Zearalenone test kit provided by the Neogen Corporation is a single-step lateral flow immunochromatographic assay based on a competitive immunoassay format. The test provides quantitative analysis for the presence of Zearalenone using a Zearalenone-antibody particle complex coated test strip and the Neogen AccuScan III and AccuScan Gold readers.

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the current policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch (PPMAB) of the Field Management Division for information on use of this test kit in official inspections including sampling, general sample preparation (e.g., grinding and dividing), reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@usda.gov.

Approved Test Kit Information

Test Kit Vendor:	<i>Neogen Corporation 800/234-5333</i>
Test Kit Name:	Reveal Q+ for Zearalenone
Product Number:	8185
Effective Date of Instructions:	07/22/2015
Instructions Revision Number	0
Conformance Range:	100 – 1000 ppb
Number of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Supplemental Analysis:	No
Approved Commodities:	Corn, distillers dried grains with solubles (DDGS), corn gluten meal, rough rice, soy flour, and wheat
Extraction method:	Shake vigorously 50 gram sample with 150 mL of 65% Ethanol/35% distilled or deionized water (v/v) for 3 minutes
Test Format:	Lateral Flow Strip
Detection Method:	AccuScan III, and AccuScan Gold Readers

PREPARATION OF TESTING MATERIALS

AccuScan III Reader Set-up

- (1) The AccuScan III reader must have software version 4.21 or newer to analyze the Reveal Q+ tests. Contact Neogen for more information.
 - a. Launch the AccuScan III program on the reader.
 - b. Select the Mycotoxin Q+ category and Q+ Zearalenone test.
 - c. Enter a sample ID (optional).
 - d. Enter the test kit lot number and A, B, C and D values from the test kit box (**for corn, DDGS and corn gluten meal use QR code for corn and for wheat, soy flour, and rough rice use QR code for wheat**) or select from the history for previously entered lot number details.

Note: Technicians must verify/update test kit lot number in the AccuScan III reader program matches the lot number of the test strips in use before testing.

- (2) Reading Test Results with the AccuScan III Reader

Test strips should be read within 1 minute after completion of the 6 minute incubation period. Refer to Reveal AccuScan III manual for detailed set-up and selection information.

- a. Fully insert the Reveal Q+ test strip into the black cartridge adapter with the sample end first and results facing out.



- b. Insert the cartridge with test strip upside-down into the reader (the test lines will face downward into the reader).



- c. The reader's green light will glow when a cartridge is inserted, and will automatically begin analyzing the cartridge.
- d. Test results will be displayed and stored in the reader.

Note: Refer to the AccuScan III manual for more detailed instructions.

AccuScan Gold Reader Set-up:



- (1) Enter the lot-specific QR code by selecting Scan QR code from the main screen.
- (2) Place the QR code into the white cartridge adapter labeled Cal/QR and insert the cartridge into the reader (**for corn, DDGS and corn gluten meal use QR code for corn and for wheat, soy flour, and rough rice use QR code for wheat**).



- (3) The valid code will be scanned by the reader and provide information on the lot number and expiration date. Verify if this information is correct and then add the lot ID to the reader by pressing “Add Lot ID”.
- (4) Return to the home screen and select the test strip icon.
- (5) Touch the mycotoxin category.
- (6) Select the Q+ for Zearalenone test type.
- (7) Ensure that the correct lot number appears on the screen for the lot that is being used.

Preparation of 1N Sodium Hydroxide (NaOH) Solution

Note: One can buy premade 1N NaOH from any commercial supplier (e.g. Sigma Aldrich catalog# 72082) or may prepare from solid sodium hydroxide pellets (Sigma Aldrich catalog# S8045) as described below:

- (1) Add slowly 4 grams of NaOH into 100 mL distilled or deionized water with stirring.
- (2) This solution should be used to adjust the pH of any sample extract that shows pH below 7.0
- (3) Label the container stating the name, date of preparation and initials of technician that prepared the solution.
- (4) Store this solution at room temperature in a tightly closed container under fume hood.

CAUTION! NaOH is corrosive. Addition of solid NaOH pellets into water is an exothermic reaction (produces heat). Stir constantly and add the NaOH slowly.

Preparation of 1N Hydrochloric (HCl) Acid Solution:

Note: One can buy premade 1N HCl from any commercial supplier (e.g. Sigma Aldrich catalog# 38283) or may prepare concentrated HCl (Sigma Aldrich catalog# 320331) as described below:

- (1) Add slowly 8.2 mL of 12.1N HCl (concentrated Hydrochloric acid) into 91.8 mL distilled or deionized water with stirring.
- (2) This solution should be used to adjust pH of any sample extract that shows pH above 8.0
- (3) Label the container stating the name, date of preparation and initials of technician that prepared the solution.
- (4) Store this solution at room temperature in a tightly closed container under a fume hood.

CAUTION! HCl is corrosive. Addition of concentrated acid into water is an exothermic reaction (produces heat). Stir constantly and add HCl slowly.

EXTRACTION PROCEDURES

- (1) **Preparation of Extraction Solvent: 65%ethanol/35%water (v/v)**
 - a. Using a 1000 mL graduated cylinder, measure 650 mL of ethanol and carefully transfer into a clean 1000 mL bottle.
 - b. Using a 500 mL graduated cylinder, measure 350 mL of distilled or deionized water and add into the bottle containing ethanol. Shake until completely mixed.
 - c. Label the container stating the mixture contained, date of preparation, and initial of the analyst who prepared the solvent.

- d. Store the solvent in a tightly closed container at room temperature until needed.

(2) **Extraction Procedures for Corn**

- a. Transfer 50 g (\pm 0.2) of ground sample into a Whirl-Pak bag.
- b. Add 150 mL of extraction solvent.
- c. Shake vigorously by mechanical shaker (250 rpm) or by hand with similar shaking action for 3 minutes.
- d. Allow the sample to settle for 1 minute. Then Filter 3 – 5 mL of the extract with a filter syringe (Neogen item #9420) into a clean sample collection tube labeled with the sample identification.
- e. After collecting the filtrate (filtered extract), dispose of the filter syringe and ground material according to waste disposal guidelines. Set the filtrate aside for sample analysis.
- f. This is the filtered extract and ready for the analysis.

(3) **Extraction Procedures for DDGS and corn gluten meal**

- a. Transfer 50 g (\pm 0.2) of ground sample into a Whirl-Pak bag.
- b. Add 300 mL of extraction solvent.
- c. Shake vigorously by mechanical shaker (250 rpm) or by hand with similar shaking action for 3 minutes.
- d. Allow the sample to settle for 1 minute. Then Filter 3 – 5 mL of the extract with a filter syringe (Neogen item #9420) into a clean sample collection tube labeled with the sample identification. Dispose the filter syringe and ground material according to waste disposal guidelines
- e. For DDGS and corn gluten meal, check the pH of the filtered extract. .
 - i. If the pH is not between 7.0 – 8.0 it needs to be adjusted. pH is typically low for these commodities.
 - ii. Using a disposable polyethylene transfer pipette, add one drop of 1N NaOH (sodium hydroxide) to the sample extract, vortex to mix, and check the pH.
 - iii. If pH is still below 7.0, add another drop of 1N NaOH, mix, and check pH again. Continue this process until the pH falls between 7.0 and 8.0, then proceed to step f.

- f. This is the pH adjusted filtered extract and ready for the analysis.

NOTE: When the test is complete, multiply the results by 2. This is necessary since twice the normal volume of 65% ethanol has been used in the extraction.

(4) Extraction Procedures for wheat, soy flour, and rough rice

- a. Transfer 50 g (\pm 0.2) of ground sample into a Whirl-Pak bag.
- b. For wheat add 250 mL of extraction solvent and for soy flour and rough rice add 300 mL of extraction solvent.
- c. Shake vigorously by mechanical shaker (250 rpm) or by hand with similar shaking action for 3 minutes.
- d. Allow the sample to settle for 1 minute. Then Filter 3 – 5 mL of the extract with a filter syringe (Neogen item #9420) into a clean sample collection tube labeled with the sample identification.
- e. After collecting the filtrate (filtered extract), dispose of the filter syringe and ground material according to waste disposal guidelines.
- f. This is the filtered extract and ready for the analysis.

NOTE: When the test is complete, multiply the results by 1.66 (for wheat) and 2 (for soy flour and rough rice)

TEST PROCEDURES

Analysis Procedure.

- (1) Place the appropriate number of red sample dilution cups and clear sample cups for each test sample in the sample cup rack. Label cups if necessary.
- (2) Using a single-channel pipettor with a new pipette tip, add 200 microliters (μ L) of sample diluent to each red sample dilution cup.
- (3) Using a new pipette tip, add 100 μ L of sample extract into each red dilution cup containing 200 μ L sample diluents. Mix by swirling with the pipette tip and then by pipetting up and down 5 times.
- (4) Transfer 100 μ L of diluted sample extract into a new clear sample cup.
- (5) Place a new Reveal Q+ for Zearalenone test strip with the sample end down into the sample cup. Start timer and incubate for 6 minutes.

- (6) At the end of the 6 minutes incubation/development period, remove the test strip from the sample cup. Read the test strip within one minute using only Neogen's Reveal AccuScan III, or AccuScan Gold Reader.

a. Reading the Results (for corn, DDGS and corn gluten meal set QR code for corn and for wheat, soy flour, and rough rice set QR code for wheat)

- (1) The strips must be immediately read using Neogen's AccuScan III, or AccuScan Gold Reader to analyze test strip. Test results will be displayed and stored in the reader.
- (2) Reading should be made between 6 and 7 minutes. Reading results after 7 minutes may be inaccurate due to over development of the device and should not be reported.
- (3) Fully inserted the Reveal Q+ test strip into the reader specific black cartridge adapter with the sample end first and results facing out.
- (4) Insert the cartridge with test strip side up into the AccuScan.
- (5) The reader will automatically begin analyzing the cartridge and will be displayed and stored in the reader.

REPORTING AND CERTIFYING TEST RESULTS

Refer to the current instructions issued by the Policies, Procedures, and Market Analysis Branch of the Field Management Division for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@usda.gov).

STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions

Store the kit components at room temperature (18-30°C, 64-86°F) to ensure full shelf life. Test strips should remain capped in their original tubes until used to ensure optimal performance.

b. Precautions

- (1) Do not use test kit components beyond the expiration date.
- (2) Test strip development times, other than those specified in Test Procedures section, may give inaccurate results.
- (3) Treat all used liquids, including sample extract, and lab ware as if contaminated with Zearalenone, gloves and other protective apparel should be worn at all times.
- (4) Ensure the device, lot number and curve details match the lot ID number selected on the reader. Failure to update the lot-specific QR code within the AccuScan reader will cause inaccurate results.

EQUIPMENT AND SUPPLIES

a. Materials provided in test kits.

- (1) 25 Reveal Q+ for Zearalenone test strips, 25 red sample dilution cups.
- (2) 25 clear sample cups, 1 bottle of sample diluent.

b. Materials required but not provided.

- (1) Timer (Neogen item #9426), 100 µL pipettor (or equivalent) with pipette tips.
- (2) Sample collection cups with lids. (Neogen item #9428),
- (3) Reveal sample rack (Neogen item #9475)
- (4) Reveal AccuScan III Reader (Neogen item #9590)
- (5) Reveal AccuScan Pro Reader (Neogen item #9565)
- (6) Reveal AccuScan God Reader (Neogen item #9595)
- (7) Disposable polyethylene transfer pipettes.
- (8) 65% Ethanol, reagent grade or better (Neogen item #8073, #8074)
- (9) Dispensing pump or graduated cylinder. (Neogen item #9448, #9447)
- (10) Filter syringe (Neogen item #9420)
- (11) Sample grinder, Scale capable of weighing 5 – 50 grams.
- (12) Bottle, 1 Liter. (Neogen item #9472)

REVISION HISTORY

- Revision 0 (07/22/2015)